Supplemental Table S1. EV Proteome

| Supplemental Ta            | able S1. EV Proteome   |          |   |              |
|----------------------------|--|----------|---|--------------|
|                            | Protein Name   |          | Average # Peptide (P. syringae infection) | B, [B], N, P |
|                            | ABC-2 and Plant PDR ABC-type transporter family protein (PEN3)                                 | 37.5     |   |              |
|                            | vacuolar ATP synthase subunit A (VHA-A)  Early-responsive to dehydration stress protein (ERD4) | 37<br>19 |   |              |
|                            | GFP-PEN1   | 18.5     |   |              |
|                            | vacuolar proton ATPase A3 (VHA-a3)   | 15.5     |   |              |
|                            | carbonic anhydrase 2 (CA2)   | 14.5     |   |              |
| AT5G14740.2<br>AT5G17920.1 | 5-methyltetrahydropteroyltriglutamatehomocysteine methyltransferase 1 (MS1)                    | 12.5     |   |              |
|                            | Pyrophosphate-energized vacuolar membrane proton pump 1 (AVP1)                                 | 12.5     |   |              |
|                            | Glyceraldehyde-3-phosphate dehydrogenase (GAPA1)   | 12.5     |   |              |
|                            | Leucine-rich repeat receptor-like protein kinase (AT3G02880)                                   | 11.5     |   |              |
|                            | V-type proton ATPase subunit E1 (VHA-E1)   | 11.5     |   |              |
|                            | Annexin D1 (ANN1)  | 11.5     |   |              |
|                            | ribulose-bisphosphate carboxylases (rbcL)  | 11       |   |              |
|                            | Hypersensitive-induced response protein 3 (HIR3)   | 11       |   |              |
|                            | V-type proton ATPase subunit C (VHA-C)   | 11       | 7.5                                       |              |
|                            | Glyceraldehyde-3-phosphate dehydrogenase (GAPC2)   | 10.5     |   |              |
|                            | Monocopper oxidase-like protein (SKU5)   | 10       | 7.5                                       |              |
| AT4G09000.1                | 14-3-3-like protein GF14 chi (GF14)  | 9.5      | 9.5                                       |              |
| AT4G02520.1                | Glutathione S-transferase F2 (GSTF2)   | 9.5      | 8   |              |
| AT2G37710.1                | L-type lectin-domain containing receptor kinase IV.1 (LECRK41)                                 | 9.5      | 3   |              |
|                            | Leucine-rich repeat receptor-like protein kinase (AT5G49760)                                   | 9        |   |              |
|                            | Early nodulin-like protein 2   | 9        |   |              |
|                            | Glycerophosphodiester phosphodiesterase (GDPDL4)   | 8        |   |              |
|                            | Tubulin beta-4 chain (TUBB4)   | 8        |   |              |
|                            | V-type proton ATPase subunit H (VHA-H)   | 8        |   |              |
| AT5G19770.1                | Tubulin alpha-3 chain (TUBA3)  | 7.5      |   |              |
|                            | Plasma-membrane associated cation-binding protein 1 (PCAP1)                                    | 7.5      |   |              |
|                            | GDSL esterase/lipase (ESM1)  | 7.5      |   |              |
|                            | Phosphoinositide phospholipase C 2 (PLC2)  | 7.5      |   |              |
|                            | Hypersensitive-induced response protein 2 (HIR2)   | 7.5      |   |              |
|                            | Phototropin 2 (PHOT2) Adapasylhomosystainasa (MEEE9)   | 7        |   |              |
|                            | Adenosylhomocysteinase (MEE58)   | 7        |   |              |
|                            | Aquaporin PIP1-2 (PIP1B) MDIS1-interacting receptor like kinase 2 (MIK2)                       | 6.5      |   |              |
|                            | WDIS1-interacting receptor like kinase 2 (WIK2) V-type proton ATPase subunit D (VHA-D)         | 6.5      |   |              |
|                            | ABC transporter B family member 4 (ABCB4)  | 6.5      |   |              |
|                            | Putative uncharacterized protein (At1g53210)   | 6.5      |   |              |
|                            | Remorin family protein (At3g61260)   | 6        |   |              |
|                            | Long chain acyl-CoA synthetase 4 (LACS4)   | 6        |   |              |
|                            | Fasciclin-like arabinogalactan protein 2 (FLA2)  | 6        |   |              |
|                            | Fasciclin-like arabinogalactan protein 8 (FLA8)  | 6        |   |              |
|                            | Elongation factor 2 (LOS1)   | 6        |   |              |
|                            | Probable calcium-binding protein (CML27)   | 6        |   |              |
|                            | Late embryogenesis abundant protein, group 2 (AT2G44060)                                       | 5.5      |   |              |
|                            | Two pore calcium channel protein 1 (TPC1)  | 5.5      |   |              |
| AT3G56190.1                | Alpha-soluble NSF attachment protein 2 (αSNAP2)  | 5.5      | 4   |              |
| AT3G46060.1                | Ras-related protein RABE1c (RABE1c)  | 5.5      | 4   | I            |
| AT3G09740.1                | Syntaxin-71 (SYP71)  | 5.5      | 4.5                                       |              |
| AT2G45820.1                | Remorin (DBP)  | 5.5      | 6.5                                       |              |
|                            | Catalase 3 (CAT3)  | 5.5      |   |              |
|                            | Unknown protein (AT2G30930)  | 5        |   |              |
|                            | Probable LRR receptor-like serine/threonine-protein kinase (AT1G53430)                         | 5        |   | 1            |
|                            | Phospholipase Dδ (PLDδ)  | 5        |   |              |
|                            | Ras-related protein RABB1c (RABB1c)  | 5        |   |              |
|                            | Triosephosphate isomerase, cytosolic (CTIMC)   | 5        |   |              |
|                            | Aquaporin PIP2-1 (PIP2-1)  | 5        |   |              |
|                            | PYK10-binding protein 1 (PBP1)   | 5        |   |              |
|                            | Bifunctional enolase 2/transcriptional activator (ENO2)  | 5        |   |              |
|                            | Protein PLANT CADMIUM RESISTANCE 1 (PCR1) Sugar Transport Protein 1 (STP1)                     | 5        |   |              |
|                            | Annexin D2 (ANN2)  |          |   |              |
|                            | Flavodoxin-like quinone reductase 1 (FQR1)   | 4.5      |   |              |
|                            | Syntaxin 132 (SYP132)  | 4.5      |   |              |
|                            | Aquaporin PIP2-7 (PIP2-7)  | 4.5      |   |              |
|                            | Probable aquaporin PIP1-5 (PIP1-5)   | 4.5      |   |              |
|                            | Cysteine synthase 1 (OASA1)  | 4.5      |   |              |
|                            | Delta(24)-sterol reductase (DIM)   | 4.5      |   |              |
|                            | V-type proton ATPase subunit G1 (VHA-G1)   | 4.5      |   |              |
|                            | Cytosolic isocitrate dehydrogenase [NADP] (CICDH)  | 4.5      |   |              |
|                            | Malate dehydrogenasee, cytoplasmic 1 (MDH1)  | 4.5      | 5   |              |
| AT1G53430.1                | Unknown protein (AT4G23670)  | 4        | 3   |              |
|                            | Uncharacterized Protein (AT1G13470)  | 4        |   |              |
|                            | Fasciclin-like arabinogalactan protein 1 (FLA1)  | 4        |   |              |
|                            | Vesicle transport v-SNARE 11 (VTI11)   | 4        |   |              |
|                            | NDR1/HIN1-Like protein 3 (NHL3)  | 4        |   |              |
|                            | Tetraspanin-18 (TOM2AH2)   | 4        |   |              |
|                            | Peptidyl-prolyl cis-trans isomerase CYP19-1 (CYP19-1)  | 4        |   |              |
|                            | Glutathione S-transferase DHAR1 (DHAR1)  | 4        |   |              |
|                            | Fasciclin-like arabinogalactan protein 9 (FLA9)  | 4        |   |              |
|                            | 1-phosphatidylinositol phosphodiesterase-related protein (AT4G38690)                           | 3.5      |   |              |
|                            | Calcium-dependent protein kinase 21 (CPK21)  | 3.5      |   |              |
|                            | Auxin-induced in root cultures protein 12 (AIR12)  | 3.5      |   |              |
|                            | Uclacyanin-2   | 3.5      |   |              |
|                            | Probable aquaporin PIP2-6 (PIP2-6)   | 3.5      |   |              |
|                            | Dehydrin ERD10 (ERD10)   | 3.5      |   |              |
|                            | Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family (AT1G17620)          | 3        |   |              |
|                            | Temperature-induced lipocalin-1 (TIL) Thiosodovin H3 (TRY2)                                    | 3        |   |              |
|                            | Thioredoxin H3 (TRX3)  | 3        |   |              |
|                            | 40S ribosomal protein S8-1 (RPS8A)   | 3        |   |              |
|                            | Protein NRT1/ PTR FAMILY 2.10 (NPF2.10)  | 3        |   |              |
|                            | Ribulose bisphosphate carboxylase/oxygenase activase (RCA)                                     | 3        |   |              |
|                            | 60S acidic ribosomal protein P2-2 (RPP2B) Dehydrin ERD14                                       | 3        |   |              |
| ~: TO\0100:T               | Denyumi Endia  | 1 3      | 1 5                                       |              |

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|----------------------------|--|------------|-------|-----|
| AT1G74050.1<br>AT1G67090.1 | 60S ribosomal protein L6-3 (RPL6C) Ribulose bisphosphate carboxylase small chain 1A (RBCS1A)   | 3          |       |     |
| AT1G07090.1<br>AT1G07890.1 | L-ascorbate peroxidase 1 (APX1)  | 3          |       |     |
| AT5G19230.1                | Uncharacterized GPI-anchored protein (At5g19230)   | 2.5        |       |     |
| AT4G36750.1                | Probable NAD(P)H dehydrogenase (quinone) FQR1-like 2 (AT4G36750)   | 2.5        |       |     |
| AT3G54200.1                | Late embryogenesis abundant hydroxyproline-rich glycoprotein (AT3G54200)   | 2.5        |       |     |
| AT1G73650.1                | Uncharacterized Protein (AT1G73650)  | 2.5        |       |     |
|                            | V-type proton ATPase subunit F (VHA-F)   | 2.5        |       |     |
|                            | RPM1-interacting protein 4 (RIN4) Sugar transport protein 4 (STP4)   | 2.5<br>2.5 |       |     |
|                            | MLP-like protein 328 (MLP328)  | 2.5        |       |     |
| AT2G26730.1                | Leucine-rich repeat receptor-like protein kinase (AT2G26730)   | 2.3        |       |     |
|                            | Blue copper protein (BCB)  | 2          |       |     |
|                            | H(+)-ATPase 2 (AHA2)   | 29         |       |     |
|                            | H(+)-ATPase 1 (AHA1)   | 15.5       |       |     |
|                            | Patellin 1 (PATL1)   | 15         | 8.5   | [B] |
|                            | Glycerophosphodiester phosphodiesterase (GDPDL3)   | 13.5       |       |     |
|                            | V-type proton ATPase subunit B2 (VHA-B2)   | 13         |       |     |
| AT3G09440.1                | Heat shock 70 kDa protein 3 (HSP70-3)  | 12.5       |       |     |
| AT1G22530.1                | Patellin 2 (PATL2)   | 10.5       | 20    |     |
|                            | Cysteine-rich receptor-like protein kinase 10 (CRK10) Heat shock cognate protein 70-1 (HSC70-1)  | 9.5<br>8.5 | 2     |     |
|                            | V-type proton ATPase subunit d1 (VHA-d1)   | 7.5        |       |     |
| AT1G70410.1                | Beta carbonic anhydrase 4 (BCA4)   | 7.5        | 4     |     |
| AT1G51805.1                | Leucine-rich repeat receptor-like protein kinase (AT1G51805)   | 6.5        |       |     |
| AT3G45780.1                | Phototropin-1 (PHOT1)  | 6.5        |       |     |
| AT2G20990.1                | Synaptotagmin A (SYTA)   | 6          |       | [B] |
| AT3G17410.1                | Putative serine/threonine protein kinase (AT3G17410)   | 5.5        | 4     | [B] |
|                            | Actin-7 (ACT7)   | 5.5        |       |     |
|                            | Nitrilase 1 (NIT1)   | 5.5        |       |     |
| AT4G35230.1                | BR-signaling kinase 1 (BSK)  | 5          |       |     |
| AT3G11130.1<br>AT3G24550.1 | Clathrin heavy chain 1 (CHC1)  Proline-rich receptor-like protein kinase 1 (PERK1)   | 5          |       |     |
| AT1G63500.1                | Protein kinase protein with tetratricopeptide repeat domain (AT1G63500)  | 3.5        |       |     |
| AT1G63500.1<br>AT1G22280.1 | Phytochrome-associated protein phosphatase type 2C (PAPP2C)  | 3.5        | 5.5   |     |
|                            | Oligopeptide transporter 3 (OPT3)  | 3.5        |       |     |
| AT4G09320.1                | Nucleoside diphosphate kinase 1 (NDK1)   | 3.5        |       |     |
| AT1G75680.1                | Endoglucanase 10   | 3.5        | 2     | [B] |
|                            | Beta-glucosidase, GBA2 type family protein (AT4G10060)   | 3          | 2     |     |
|                            | Aspartyl protease family protein (AT3G51330)   | 3          |       |     |
| AT2G34510.1                | Putative uncharacterized protein (At2g34510)   | 3          |       |     |
| AT1G22300.1                | 14-3-3-like protein GF14 epsilon (GRF10)   | 3          |       |     |
| AT5G57110.1                | Calcium-transporting ATPase 8 (ACA8)   | 3          | _     |     |
|                            | Patatin-like protein 1 (PLP1)  | 3          |       |     |
| AT3G63520.1<br>AT3G15730.1 | Carotenoid 9,10(9',10')-cleavage dioxygenase 1 (CCD1) Phospholipase D \( \pi \) 1 (PLD\( \pi \))   | 3          |       |     |
|                            | Glutathione S-transferase F10 (GSTF10)   | 3          |       |     |
|                            | Glycine-rich RNA-binding protein 7 (CCR2)  | 3          | 1.5   |     |
| AT2G20630.1                | Probable protein phosphotase induced by AVRRPM1 (PPC2)   | 3          |       |     |
| AT1G43170.1                | 60S ribosomal protein L3-1 (RP1)   | 3          | 2.5   | [B] |
|                            | Uncharacterized GPI-anchored protein (At5g19240)   | 2.5        |       |     |
| AT4G39730.1                | Lipase/lipooxygenase, PLAT/LH2 family protein  | 2.5        |       |     |
|                            | Patched family protein   | 2.5        |       |     |
|                            | Heat shock protein 90-4 (HSP90-4)  | 2.5        |       |     |
| AT5G45750.1<br>AT5G11670.1 | Ras-related protein RABA1c (RABA1c) NADP-dependent malic enzyme 2 (NADP-ME2)   | 2.5<br>2.5 |       |     |
| AT2G31880.1                | Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase (SOBIR1)  | 2.5        |       |     |
| AT1G02920.1                | Glutathione S-transferase F7 (GSTF7)   | 2.5        | 4     |     |
| AT4G29900.1                | Calcium-transporting ATPase 10, plasma membrane-type (ACA10)   | 2.5        |       |     |
| AT4G13510.1                | Ammonium transporter 1 member 1 (AMT1-1)   | 2.5        | 1.5   |     |
| AT4G11850.1                | Phospholipase D γ1 (PDLδ1)   | 2.5        | 1     | [B] |
|                            | Protein NRT1/ PTR FAMILY 8.1 (NPF8.1)  | 2.5        |       |     |
|                            | Syntaxin-122 (SYP122)  | 2.5        |       |     |
|                            | 60S ribosomal protein L8-1 (RPL8A)   | 2.5        |       |     |
|                            | Fasciclin-like arabinoogalactan protein 7 (FLA7) Involved in response to salt stress (AT1G13930)   | 2.5<br>2.5 |       |     |
|                            | Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein (AT1G55260)  | 2.5        |       |     |
|                            | Similar to Pto kinase interactor 1 (AT1G48210)   | 2          |       |     |
| AT5G44130.1                | Fasciclin-like arabinoogalactan protein 13 (FLA13)   | 2          |       |     |
|                            | Glycerophosphodiester phosphodiesterase (GDPDL1)   | 2          |       | [B] |
| AT5G54110.1                | Vesicle-associated protein 4-1 (PVA41)   | 2          |       | [B] |
| AT4G34950.1                | Major facilitator family protein (AT4G34950)   | 2          |       |     |
| AT4G31140.1                | Glucan endo-1,3-beta-glucosidase 5   | 2          |       |     |
|                            | 60S ribosomal protein L24-2 (RPL24B)   | 2          |       |     |
| AT3G28450.1<br>AT3G09820.1 | Inactive LRR receptor-like serine/threonine-protein kinase (BIR2)  Adenosine kinase 1 (ADK1)   | 2          |       |     |
|                            | 60S acidic ribosomal protein P0-2 (RPP0B)  | 2          |       |     |
|                            | MLP-like protein 43 (MLP43)  | 2          |       |     |
| AT1G61250.1                | secretory carrier-associated membrane protein 3 (SC3)  | 2          |       |     |
| AT1G52290.1                | Proline-rich receptor-like protein kinase 15 (PERK15)  | 2          |       | [B] |
|                            | Triacylglycerol lipase-like 1 (TLL1)   | 2          |       |     |
| AT1G27950.1                | Non-specific lipid transfer protein GPI-anchored 1 (LTPG1)   | 2          |       |     |
|                            | Protein PLANT CADMIUM RESISTANCE 2 (PCR2)  | 2          |       |     |
| ATECES000.1                | Lipase/lipooxygenase, PLAT/LH2 family protein (AT2G22170)  | 1.5        |       |     |
| AT5G58090.1<br>AT5G26340.1 | Glucan endo-1,3-beta-glucosidase 6 Sugar transport protein 13 (STP13)  | 1.5<br>1.5 |       |     |
|                            | Vegetative storage protein 13 (SIP13)  | 1.5        |       |     |
| AT4G23650.1                | Calcium-dependent protein kinase 3 (CPK3)  | 1.5        |       |     |
|                            | 60S ribosomal protein L18-2 (RPL188)   | 1.5        |       |     |
|                            | Glutathione S-transferase F9 (GSTF9)   | 1.5        | 2.5   | [B] |
| AT1G75220.1                | Sugar transporter ERD6-like 6  | 1.5        | 2.5   | [B] |
|                            | la entre de la companya del companya del companya de la companya d | 1          | 2.5   | [B] |
|                            | Lysine histidine transporter 1 (LHT1)  |            |       |     |
| AT4G23600.1                | Cystine lyase (CORI3)  | 1          | 7     |     |
| AT4G23600.1<br>AT2G31570.1 |  |            | 7 2.5 | [B] |

| AT1G09070.1 | Soybean gene regulated by cold-2 homolog (SRC2)                         | 1    | 2   | [B] |
|-------------|---|------|-----|-----|
| AT3G16470.1 | Jacalin-related lectin 35 (JAL35)                                       | 0.5  | 5   | [B] |
| AT2G39330.1 | Jacalin-related lectin 23 (JAL23)                                       | 0.5  | 8   | [B] |
| AT2G20450.1 | 60S ribosomal protein L14-1 (RPL14A)                                    | 0.5  | 2.5 | [B] |
| AT1G45145.1 | Thioredoxin H5 (TRX5)   | 0.5  | 2   | [B] |
| AT1G20260.1 | V-type proton ATPase subunit B3 (VHA-B3)                                | 23.5 | 0   | N   |
| AT3G60750.1 | Transketolase-1 (TKL-1)   | 4    | 0   | N   |
| AT1G07410.1 | Ras-related protein RABA2b (RABA2b)                                     | 3.5  | 0   | N   |
| AT3G61050.1 | Calcium-dependent lipid-binding (CaLB domain) family protein (AT3G6105) | 2.5  | 0   | N   |
| AT1G56190.1 | Phosphoglycerate kinase family protein (AT1G56190)                      | 2.5  | 0   | N   |
| AT5G06530.1 | ABC transporter G family member 22 (ABCG22)                             | 2    | 0   | N   |
| AT1G23310.1 | Glutamateglyoxylate aminotransferase 1 (GGAT1)                          | 2    | 0   | N   |
| AT1G09310.1 | Putative uncharacterized protein (AT1G09310)                            | 2    | 0   | N   |
| AT3G08530.1 | Clathrin heavy chain 2 (CHC2)   | 0    | 7.5 | P   |
| AT2G39518.1 | CASP-like protein 4D2   | 0    | 2   | P   |
| AT2G37180.1 | Aquaporin PIP2-3 (PIP2-3)   | 0    | 2.5 | P   |
|             |   |      |     |     |

Mass spectrometry analysis was performed on density-gradient purified EVs from uninfected or *P. syringae*-infected GFP-PEN1 plants (two replicates per treatment). 170 EV-associated proteins from uninfected GFP-PEN1 plants and 142 EV-associated proteins from *P. syringae*-infected GFP-PEN1 plants were chosen for further analysis; these proteins were identified in both replicates of a given treatment with at least two independent peptides in each replicate. Proteins were labeled "B," "[B]," "N" or "P" depending on their presence under different treatments. Proteins labeled "B" were associated with EVs under both treatments. Proteins labeled "B" were only associated with EVs from uninfected plants, while proteins labeled "P" were only associated with EVs from *P. syringae*-infected plants,